Variation in Leaf Anatomical Characters in Response to Air Pollution in Some Euphorbiaceae Species

S. E. Ekpemerechi[1], A. A. Ajao[1], M. A. Jimoh[2] and S. A. Saheed[1]*
[1]*Obafemi Awolowo University, Department of Botany, Ile-Ife, 220005, Nigeria.*
[2]*Osun State University, Department of Biological Sciences, Osogbo, Nigeria.*
*Corresponding Author: *saheed@oauife.edu.ng*

Abstract

Dry and wet season studies of the leaf anatomy of ten plant species in the family Euphorbiaceae from three sites with different pollution levels in Southwestern Nigeria were carried out. This is with a view to establish the response of plant anatomical structures to air pollution. The species investigated were *Alchornea laxiflora* (Benth.) Pax & K. Hoffm., *A. cordifolia* (Schum. & Thonn.) Mull. Arg., *Euphorbia heterophylla* L., *E. hyssopifolia* L., *E. hirta* L., *Croton lobatus* L., *Flueggea virosa* (Willd) Voigt., *Manihot esculenta* Crantz., *Phyllanthus amarus* (Schum. & Thonn.) and *Acalypha ornata* Hochst. ex A. Rich. Standard anatomical procedures for examining leaf epidermal surfaces and cross sections of the leaves were employed. Epidermal characters such as stomatal number and stomatal size were investigated while other leaf tissues including thicknesses of the cuticles, thickness of the epidermis as well as palisade and spongy mesophyll tissues thickness. Our data revealed a significant ($P < 0.05$) reduction in all the examined anatomical characters in plants growing in polluted sites when compared with their counterparts from unpolluted environments, except for stomatal number which showed a corresponding significant ($P < 0.05$) increase. Changes in the anatomical characters reported in this study tend to corroborate responses observed in the morphological attributes of the same plant species to environmental pollution in an earlier report. This study further establishes the usefulness of anatomical studies in explaining the mechanism underlying the morphological responses of the plants to air pollution.

Introduction

Global advancement in industrialization, urbanization as well as economic growth is generally associated with increased demand for energy particularly from fossil fuels, which results in increased emission of toxic gases and other substances to the environment (Kalandadze, 2003; Uaboi-Egbenni *et al.,* 2009). Pollution due to toxic gaseous emissions engenders deleterious effects on plants, especially those growing in many urban areas (Qadir & Iqbal, 1991; Giri *et al.,* 2013). Therefore, studies on acclimation of plants such as these are important to afford a better understanding of the modifications that occur in relation to changes in environmental conditions.

Plants growing in urban areas have been reported to be greatly affected by pollutants such as oxides of nitrogen, sulphur dioxide, hydrocarbons, ozone, particulate matter, peroxyacyl nitrates (PAN) among others (Jahan & Iqbal, 1992). These effects are associated with alteration of specific morphological, anatomical, physiological and biochemical attributes of the plant (Kovacic & Nikolic, 2005; Pandey *et al.,* 2006). Studies have been carried out on the

effects of air pollution on the internal tissues (anatomy) of plant species in different families (see Ninoval *et al.,* 1983; Iqbal, 1985; Sodnik *et al.* 1987; Gupta & Ghouse, 1988; Jahan & Iqbal, 1992; Ivanescu & Gostin, 2007; Stevovic *et al*., 2010). From the structural point of view, the plants from polluted sites experience modifications to their tissues, largely with respect to reduction in thickness of palisade parenchyma, spongy mesophyll, epidermis and cuticles. For example, Iqbal (1985) as well as Jahan & Iqbal (1992) showed significant reduction in thickness of the cuticle, epidermis, hypodermis, palisade and spongy parenchyma cells in polluted leaves as compared to leaves collected from non-polluted areas. Recently, Stevovic et al. (2010) reports that Tansy plants grown in polluted areas showed reduction in the thicknesses of mesophyll parenchyma, upper and lower epidermal tissues.

Emberson *et al.* (2001) reported an exciting data relating to the impact of air pollution on vegetation especially in developing countries. A previous paper by Fenger, (1999) observed that most attention on this type of studies are focused on the effects of these emission on human health. Those that were directed to plants are usually from developed countries especially Europe and North America (Heck *et al.*, 1983; Fuhrer *et al.,* 1997). Equally interesting is the fact that data collated by Emberson et al. (2001) from Africa are only from Egypt and South Africa. There was no data from Nigeria which reportedly is a fast-growing economy, in their report to showcase responses of the diverse vegetation cover to varying pollution levels experienced there.

This study aimed at establishing specific response of internal tissues of some plant species in the family Euphorbiaceae to varying levels of air pollution with a view to possibly explain the mechanism underlying the observed structure-function response of plants to air pollution. A recent study by Ekpemerechi *et al.* (2014) reported the foliar morphological response of different plant species in the family Euphorbiaceae to air pollution. The current study is a follow-up to that study as it examine changes in anatomical characters of these species in response to air pollution which possibly may showcase the mechanism behind the reported morphological response. The choice of these species in the Euphorbiaceae is because it is a large family whose species are widely distributed in the tropics, sub-tropics and temperate regions (Watson & Dallwitz, 1992; Olorode, 2012). This significant attribute of the Euphorbiaceae indicates high ecological plasticity and adaptability of its species to different environment conditions.

Materials and methods

Study area

The study was carried out in three different sites with established varying level of air pollution (Ekpemerechi *et al*., 2014) in South-Western Nigeria viz: (i) a rural area (control site) along Tonkere village, after Road 8, Obafemi Awolowo University, Ile-Ife (N 07^0 32.243'; E004^0 31.121'), (ii) a sub-urban area close to the Toll Gate at the Ile-Ife end of the Ile-Ife – Ibadan highway

(N 07^0 29.601'; E 004^0 29.483') and (iii) an urban area close to Toll Gate at the Ibadan end of the Ibadan – Lagos highway (N 07^0 19.201'; E 003^0 56.400'). The sites for collection of plant samples were located approximately 50m away from these roads.

Plant materials

A reconnaissance survey into the study sites revealed species that are most frequently encountered. Ten of such species in the family Euphorbiaceae were selected for this study. They include *Alchornea laxiflora* (Benth.) Pax & K. Hoffm., *Alchornea cordifolia* (Schum. & Thonn.) Mull. Arg., *Euphorbia heterophylla* L., *Euphorbia hyssopifolia* L., *Euphorbia hirta* L., *Croton lobatus* L., *Flueggea virosa* (Willd) Voigt., *Manihot esculenta* Crantz., *Phyllanthus amarus* (Schum. & Thonn.) and *Acalypha ornata* Hochst. ex A. Rich. Fresh leaves at the same leaf stage occurring at the same level of insertion on the stem were collected for each species from the three study sites. Attention was given to plants that were closer to the traffic pathway with direct contact with automobile exhaust emission. The plant sample collections were done during wet and dry seasons.

Anatomical study

For the purpose of studying the detailed anatomical structures, sizeable portions of the leaves were cut from the standard median parts of the mature and well expanded leaves (i.e. mid-way between the base and the apex) for each species. For the study of stomata number and stomatal size, epidermal peels of the leaf were obtained manually, while materials difficult to process manually were obtained using concentrated nitric acid following standard procedures previously described (Adedeji & Jewoola, 2008; Ogundare & Saheed, 2012). For the study of internal tissues of the leaf, transverse sections of the leaves were cut at a thickness of 20m using Reichert sliding microtome. Specimens were processed using standard anatomical procedures as described previously (Illoh, 1995; Saheed & Illoh, 2010). All microscopic measurements were made with the aid of an ocular micrometer inserted in the eyepiece of the Celestron compound microscope fitted with a JVC KYF70B digital camera. These measurements were later multiplied by the ocular constant with respect to the objectives under which they were taken.

Data analyses

For each parameter, 20 measurements were made and the data were analyzed using a one way analysis of variance to test for significant differences in the observed effects of the pollutants on the parameters considered across the locations at the probability level of $P < 0.05$. The significant means were separated using a post-hoc test. SPSS statistical package Model 9.0 was used for the analysis.

Results

Data presented are on the quantitative description of various anatomical characters of the plant species studied.

Leaf epidermal surface. In general, results show that in all the species investigated

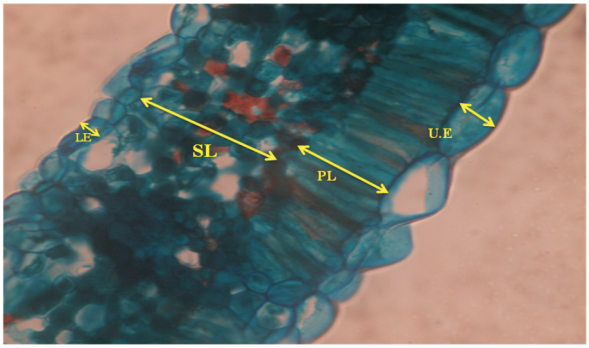

Fig 1. A typical transverse section of the leaf of *A. laxiflora* showing anatomical features of interest. Scale = 25µm

for both dry and wet seasons, stomata number significantly ($P < 0.05$) increased from the rural to the sub-urban and to the urban areas (Tables 1 and 2). For example, on the adaxial surface in dry season, there is a significant increase ($P < 0.05$) in the number of stomata in *A. laxiflora* from 0.38 ± 0.92µm in the rural area to 1.13±1.28µm in the sub-urban and 1.75 ± 1.18 µm in urban area. However, the stomatal size was generally found to reduce significantly ($P < 0.05$) from rural to sub-urban and to the urban areas on both surfaces and in both seasons (Tables 3 and 4). For example, in dry season and on the adaxial surface, *E. hirta* showed a significant reduction ($P < 0.05$) in the size of stomata from 111.60 ± 0.52µm in the rural, to 43.00 ± 0.14µm in the sub-urban and 18.07 ± 0.12µm in urban area. Other data for the size of the stomata are as presented in Tables 3 and 4 for dry and wet seasons respectively.

Leaf transverse section

Differences in the thickness of various characters of the leaf anatomy such as cuticle, epidermis, palisade and spongy mesophyll were obtained for both dry and wet seasons (Tables 5 and 6). There is a significant ($P < 0.05$) reduction generally in the thickness of these characters from the rural to the sub-urban and to the urban areas and across the two seasons investigated. For example, in dry season, the upper cuticle of *A. cordifolia* significantly reduced ($P < 0.05$) across the three study sites (Table 5) with a mean thickness of 1.48 ± 0.41 µm (rural), 1.40 ± 0.38 µm (sub-urban) and 1.08 ± 0.24 µm (urban). In a similar manner for upper epidermis in dry season, *C. lobatus* showed a significant reduction ($P < 0.05$) in the mean thickness of the upper epidermis from the rural location with 5.20 ± 0.69 µm to sub-urban with 3.35 ± 0.75 µm to urban with 1.75 ± 0.44 µm (Table 5). This same trend was observed for palisade mesophyll cells in dry season where for example in *F. virosa* there is significant reduction ($P < 0.05$) in the thickness of palisade mesophyll from the rural area with mean thickness of 23.60±2.09 µm to the sub-urban with a value of 21.15 ± 1.35 µm and urban with 17.05 ± 1.70 µm in mean thickness (Table 5).

The thickness of spongy mesophyll followed a similar pattern as others, reducing significantly from rural to sub-urban and eventually to urban area. For example in the wet season, the thickness in *M. esculenta* showed a significant reduction ($P < 0.05$) from 28.30 ± 1.69 µm in the rural to 26.05 ± 4.07 µm in the sub-urban and 21.75 ± 3.09 µm in the urban area. The other two characters (lower epidermis and cuticle) investigated equally followed a similar trends as those

TABLE 1

Size of the stomata opening on epidermal surfaces of the plant species studied.

	Dry season *Number of stomata/Unit area(μm²)*					
	Adaxial surface			*Abaxial surface*		
Plant species	*Urban*	*S.urban*	*Rural*	*Urban*	*S.Urban*	*Rural*
A. laxiflora	1.75 ± 1.18^{a}	1.13 ± 1.28^{b}	0.38 ± 0.92^{c}	5.75 ± 1.83^{a}	2.38 ± 1.51^{b}	0.75 ± 1.18^{c}
A. cordifolia	7.50 ± 1.40^{a}	4.75 ± 1.60^{b}	3.25 ± 1.18^{c}	10.25 ± 2.0^{a}	5.88 ± 2.19^{b}	4.25 ± 2.00^{c}
E. heterophylla	9.75 ± 2.91^{a}	8.38 ± 3.27^{b}	5.13 ± 2.22^{c}	4.25 ± 2.58^{a}	2.25 ± 1.38^{b}	0.88 ± 1.22^{c}
E. hyssopifolia	11.38 ± 2.8^{a}	7.25 ± 2.28^{b}	3.50 ± 1.26^{c}	7.25 ± 2.13^{a}	2.50 ± 0.00^{b}	4.00 ± 1.26^{c}
E. hirta	8.13 ± 1.60^{a}	5.75 ± 1.64^{b}	3.88 ± 1.28^{c}	7.13 ± 2.03^{a}	2.88 ± 1.86^{b}	1.75 ± 2.00^{c}
C. lobatus	8.75 ± 1.72^{a}	4.88 ± 1.90^{b}	3.13 ± 1.11^{c}	12.75 ± 2.9^{a}	10.13 ± 1.7^{b}	6.00 ± 1.26^{c}
F. virosa	26.50 ± 4.6^{a}	14.13 ± 2.8^{b}	9.88 ± 2.06^{c}	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
M. esculenta	4.63 ± 2.03^{a}	2.05 ± 1.62^{b}	13.00 ± 2.2^{c}	15.13 ± 3.5^{a}	11.25 ± 3.1^{b}	10.13 ± 2.06^{c}
P. amarus	7.50 ± 2.29^{a}	4.63 ± 1.86^{b}	2.13 ± 1.86^{c}	9.50 ± 2.99^{a}	6.38 ± 1.72^{b}	2.86 ± 1.86^{c}
A. ornata	8.75 ± 2.07^{a}	5.63 ± 2.28^{b}	–	8.63 ± 2.86^{a}	7.38 ± 2.63^{b}	–

Values are means ± standard deviation, n = 20, similar letters on tops of values across the row indicate values not significantly different at $P < 0.05$ for each species, – = values not determined.

TABLE 2

Number of stomata on the epidermal surfaces of the plant species studied.

	Wet season Number of stomata/Unit area(μm²)					
	Adaxial surface			Abaxial surface		
Plant species	Urban	S.urban	Rural	Urban	S.urban	Rural
A. laxiflora	3.88 ± 1.28^{a}	2.13 ± 1.22^{b}	1.13 ± 1.28^{c}	7.75 ± 1.60^{a}	2.88 ± 1.68^{b}	1.75 ± 1.64^{c}
A. cordifolia	8.13 ± 1.97^{a}	7.00 ± 1.74^{b}	3.63 ± 1.28^{c}	15.25 ± 3.13^{a}	12.88 ± 3.17^{b}	9.76 ± 2.28^{c}
E. heterophylla	9.25 ± 3.35^{a}	6.00 ± 1.70^{b}	3.88 ± 2.06^{c}	8.00 ± 2.24^{a}	6.00 ± 1.88^{b}	4.00 ± 2.49^{c}
E. hyssopifolia	10.00 ± 1.40^{a}	4.38 ± 2.42^{b}	–	9.25 ± 2.00^{a}	6.63 ± 2.19^{b}	
E. hirta	11.75 ± 2.83^{a}	8.75 ± 1.72^{b}	5.00 ± 2.43^{c}	11.75 ± 1.18^{a}	10.38 ± 1.22^{b}	8.25 ± 1.43^{c}
C. lobatus	11.25 ± 1.28^{a}	6.15 ± 1.14^{b}	–	7.88 ± 1.47^{a}	3.63 ± 2.63^{b}	–
F. virosa	34.75 ± 2.13^{a}	17.00 ± 2.24^{b}	11.63 ± 1.47^{c}	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
M. esculenta	13.60 ± 1.53^{a}	7.50 ± 1.40^{b}	5.99 ± 0.91^{c}	15.50 ± 1.74^{a}	9.63 ± 1.68^{b}	4.88 ± 1.51^{c}
P. amarus	16.13 ± 2.86^{a}	12.38 ± 2.22^{b}	7.25 ± 1.80^{c}	17.88 ± 3.06^{a}	13.75 ± 2.22^{b}	7.63 ± 1.51^{c}
A. ornata	14.75 ± 1.80^{a}	9.88 ± 1.72^{b}	–	13.63 ± 1.28^{a}	10.88 ± 2.19^{b}	–

Values are means± standard deviation, n = 20, similar letters on tops of values across the row indicate values not significantly different at $P < 0.05$ for each species, – = values not determined.

TABLE 3
Size of the stomata opening on epidermal surfaces of the plant species studied.

	Dry season size of stomata opening (µm²)					
	Adaxial surface			Abaxial surface		
Plant species	Urban	S.urban	Rural	Urban	S.urban	Rural
A. laxiflora	29.38 ± 12.2^{a}	54.38 ± 23.2^{b}	107.8 ± 28.3^{c}	18.72 ± 3.7^{a}	51.10 ± 17.63^{b}	74.06 ± 9.34^{c}
A. cordifolia	19.33 ± 8.38^{a}	39.50 ± 4.58^{b}	70.13 ± 8.39^{c}	8.66 ± 0.19^{a}	35.61 ± 15.81^{b}	68.03 ± 0.35^{c}
E. heterophylla	8.60 ± 0.24^{a}	34.99 ± 0.30^{b}	72.36 ± 0.20^{c}	8.67 ± 0.22^{a}	52.46 ± 0.21^{b}	52.68 ± 0.27^{c}
E. hyssopifolia	15.01 ± 0.13^{a}	41.66 ± 0.22^{b}	102.31 ± 0.5^{c}	14.91 ± 0.6^{a}	41.41 ± 0.43^{b}	102.33 ± 0.61^{c}
E. hirta	18.07 ± 0.12^{a}	43.00 ± 0.14^{b}	111.6 ± 0.52^{c}	36.88 ± 0.0^{a}	53.75 ± 0.00^{b}	101.38 ± 0.00^{c}
C. lobatus	18.44 ± 4.2^{a}	43.52 ± 10.3^{b}	67.81 ± 14.1^{c}	22.47 ± 5.3^{a}	48.79 ± 9.54^{b}	112.39 ± 9.48^{c}
F. virosa	58.13 ± 8.1^{a}	63.56 ± 12.2^{b}	132.41 ± 9.5^{c}	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
M. esculenta	11.25 ± 4.58^{a}	$33.\pm 11.92^{b}$	67.57 ± 8.89^{c}	67.07 ± 8.5^{a}	90.45 ± 0.51^{b}	117.59 ± 13.96^{c}
P. amarus	15.57 ± 1.33^{a}	46.14 ± 0.45^{b}	70.93 ± 0.50^{c}	15.03 ± 0.5^{a}	39.47 ± 6.98^{b}	79.06 ± 0.65^{c}
A. ornata	27.09 ± 0.82^{a}	60.14 ± 11.7^{b}	–	46.22 ± 0.2^{a}	78.40 ± 8.27^{b}	–

Values are means ± standard deviation, n = 20, similar letters on tops of values across the row indicate values not significantly different at $p < 0.05$ for each species, – = values not determined.

TABLE 4
Size of the stomata opening on epidermal surfaces of the plant species studied.

	Wet season Size of stomata opening(µm²)					
	Adaxial surface			Abaxial surface		
Plant species	urban	S.urban	Rural	Urban	S.urban	Rural
A. laxiflora	47.97 ± 20.6^{a}	77.34 ± 17.1^{b}	111.56 ± 34.3^{c}	45.64 ± 12.6^{a}	73.44 ± 17.27^{b}	112.01 ± 23.51^{c}
A. cordifolia	42.97 ± 15.0^{a}	65.08 ± 17.9^{b}	126.35 ± 27.0^{c}	37.46 ± 3.56^{a}	72.50 ± 19.62^{b}	143.13 ± 20.07^{c}
E. heterophylla	40.02 ± 0.38^{a}	72.44 ± 0.40^{b}	109.71 ± 16.4^{c}	40.05 ± 0.42^{a}	72.19 ± 0.67^{b}	109.06 ± 16.40^{c}
E. hyssopifolia	36.65 ± 0.44^{a}	91.31 ± 0.46^{b}	–	41.38 ± 0.34^{a}	90.29 ± 0.47^{b}	–
E. hirta	30.54 ± 0.30^{a}	58.50 ± 0.65^{b}	143.16 ± 0.20^{c}	58.75 ± 0.00^{a}	93.13 ± 0.00^{b}	129.50 ± 0.00^{c}
C. lobatus	52.83 ± 0.44^{a}	87.46 ± 0.21^{b}	–	65.00 ± 26.6^{a}	121.15 ± 0.15^{b}	–
F. virosa	50.0 ± 18.96^{a}	86.46 ± 6.43^{b}	162.50 ± 0.00^{c}	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
M. esculenta	38.91 ± 12.9^{a}	59.18 ± 5.51^{b}	102.53 ± 0.55^{c}	90.44 ± 0.38^{a}	117.75 ± 14.0^{b}	197.47 ± 0.56^{c}
P. amarus	37.20 ± 2.84^{a}	68.43 ± 0.55^{b}	124.65 ± 1.02^{c}	39.50 ± 6.95^{a}	71.11 ± 0.40^{b}	142.29 ± 0.72^{c}
A. ornata	51.76 ± 2.80^{a}	110.24 ± 0.3^{b}	–	77.34 ± 26.5^{a}	149.10 ± 0.55^{b}	–

Values are means± standard deviation, n=20, similar letters on tops of values across the row indicate values not significantly different at $p<0.05$ for each species, — = values not determined.

TABLE 5
Thickness of the leaf anatomical characters of the plant species studied.

Dry season		U.C (μm)	U.E(μm)	P(μm)	S(μm)	L.E(μm)	L.C(μm)	
A. laxiflora	*Urban*	1.00 ± 0.00^{a}	1.58 ± 0.34^{a}	9.84 ± 0.37^{a}	11.89 ± 1.76^{a}	1.29 ± 0.49^{a}	0.58 ± 0.18^{a}	
	Sub Urban	1.33 ± 0.33^{b}	2.45 ± 0.50^{b}	14.29 ± 1.10^{b}	16.24 ± 2.74^{b}	2.14 ± 1.06^{b}	0.93 ± 0.41^{b}	
	Rural	1.65 ± 0.46^{b}	2.62 ± 0.43^{b}	18.83 ± 2.02^{c}	19.45 ± 2.54^{c}	2.30 ± 0.39^{c}	1.20 ± 0.34^{c}	
A. cordifolia	*Urban*	1.08 ± 0.24^{a}	2.48 ± 0.64^{a}	18.90 ± 1.96^{a}	20.80 ± 4.31^{a}	1.10 ± 0.31^{a}	1.00 ± 0.00^{a}	
	Sub Urban	1.40 ± 0.38^{b}	2.83 ± 0.49^{b}	23.20 ± 2.35^{b}	24.35 ± 3.05^{b}	2.10 ± 0.55^{b}	1.33 ± 0.41^{b}	
	Rural	1.48 ± 0.41^{c}	3.15 ± 0.49^{c}	23.95 ± 2.48^{c}	27.40 ± 2.06^{c}	2.80 ± 0.41^{c}	1.70 ± 0.47^{c}	
E. heterophylla	*Urban*	1.00 ± 0.00^{a}	3.73 ± 0.79^{a}	13.50 ± 1.70^{a}	20.40 ± 2.89^{a}	2.35 ± 0.81^{a}	1.00 ± 0.00^{a}	
	Sub Urban	1.40 ± 0.50^{b}	5.00 ± 0.00^{b}	26.75 ± 1.41^{b}	24.30 ± 2.56^{b}	2.93 ± 1.03^{a}	1.20 ± 0.25^{b}	
	Rural	1.85 ± 0.37^{b}	6.50 ± 0.00^{c}	27.62 ± 1.13^{c}	28.60 ± 1.79^{c}	4.56 ± 0.51^{c}	2.00 ± 0.00^{c}	
E. hyssopifolia	*Urban*	1.00 ± 0.00^{a}	4.70 ± 0.47^{a}	11.20 ± 3.05^{a}	9.50 ± 2.80^{a}	2.50 ± 0.51^{a}	1.15 ± 0.37^{a}	
	Sub Urban	1.40 ± 0.50^{b}	5.00 ± 0.97^{b}	16.05 ± 2.37^{b}	19.05 ± 2.67^{b}	5.55 ± 0.51^{b}	1.40 ± 0.50^{b}	
	Rural	1.40 ± 0.50^{c}	5.91 ± 1.33^{c}	29.90 ± 0.31^{c}	25.20 ± 1.79^{c}	7.35 ± 0.67^{c}	2.30 ± 0.47^{c}	
E. hirta	*Urban*	1.00 ± 0.00^{a}	3.45 ± 0.10^{a}	10.30 ± 1.56^{a}	21.80 ± 1.70^{a}	1.65 ± 0.42^{a}	1.0 ± 0.17^{a}	
	Sub Urban	1.57 ± 0.29^{b}	4.80 ± 0.77^{b}	17.50 ± 3.09^{b}	21.85 ± 3.47^{b}	2.70 ± 0.57^{b}	1.45 ± 0.51^{b}	
	Rural	2.17 ± 0.18^{c}	7.20 ± 0.95^{c}	19.55 ± 1.64^{c}	27.25 ± 1.68^{c}	3.75 ± 0.64^{c}	1.90 ± 0.27^{b}	
C. lobatus	*Urban*	1.00 ± 0.00^{a}	1.75 ± 0.44^{a}	15.00 ± 0.11^{a}	18.95 ± 2.19^{a}	2.20 ± 0.41^{a}	1.00 ± 0.00^{a}	
	Sub Urban	1.30 ± 0.38^{b}	3.35 ± 0.75^{b}	20.30 ± 1.59^{b}	21.35 ± 3.73^{b}	2.55 ± 0.60^{a}	1.33 ± 0.37^{b}	
	Rural	1.48 ± 0.50^{c}	5.20 ± 0.69^{c}	28.00 ± 1.95^{c}	26.30 ± 2.49^{c}	3.25 ± 0.91^{c}	2.00 ± 0.40^{c}	
F. virosa	*Urban*	1.20 ± 0.41^{a}	4.35 ± 0.59^{a}	17.05 ± 1.70^{a}	19.25 ± 1.45^{a}	1.50 ± 0.51^{a}	1.20 ± 0.07^{a}	
	Sub Urban	2.05 ± 0.39^{b}	5.30 ± 0.98^{b}	21.15 ± 1.35^{b}	22.55 ± 2.11^{b}	2.00 ± 0.00^{a}	1.50 ± 0.26^{b}	
	Rural	3.35 ± 1.18^{c}	6.10 ± 1.17^{c}	23.60 ± 2.09^{c}	25.05 ± 4.57^{c}	2.43 ± 0.26^{c}	3.15 ± 0.37^{c}	
M. esculenta	*Urban*	1.55 ± 0.51^{a}	2.85 ± 0.75^{a}	19.75 ± 1.77^{a}	12.25 ± 2.38^{a}	1.50 ± 0.61^{a}	1.03 ± 0.12^{a}	
	Sub Urban	1.95 ± 0.39^{b}	3.50 ± 0.69^{b}	22.70 ± 1.75^{b}	15.05 ± 2.76^{b}	3.30 ± 0.47^{b}	1.65 ± 0.46^{b}	
	Rural	2.35 ± 0.49^{c}	4.50 ± 1.06^{c}	26.45 ± 1.28^{c}	20.95 ± 2.21^{c}	3.50 ± 0.61^{c}	1.77 ± 0.24^{c}	
P. amarus	*Urban*	1.00 ± 0.00^{a}	1.28 ± 0.24^{a}	12.85 ± 2.01^{a}	16.95 ± 1.96^{a}	2.40 ± 0.50^{a}	1.00 ± 0.00^{a}	
	Sub Urban	1.30 ± 0.38^{b}	2.50 ± 0.43^{b}	19.55 ± 1.76^{b}	21.40 ± 2.49^{b}	2.90 ± 0.97^{b}	1.40 ± 0.50^{b}	
	Rural	2.00 ± 0.00^{c}	3.70 ± 0.47^{c}	21.15 ± 2.50^{b}	23.4 ± 2.26^{bc}	4.00 ± 0.00^{c}	2.00 ± 0.00^{b}	
A. ornata	*Urban*	1.20 ± 0.00^{a}	3.05 ± 0.89^{a}	17.40 ± 1.90^{a}	17.80 ± 3.11^{a}	2.40 ± 0.75^{a}	1.05 ± 0.22^{a}	
	Sub Urban	1.55 ± 0.51^{b}	3.45 ± 0.51^{b}	17.90 ± 2.17^{b}	18.90 ± 3.57^{b}	4.05 ± 0.60^{b}	1.37 ± 0.50^{b}	
	Rural	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	

Values are means± standard deviation, n = 20, similar letters on tops of values along the column indicate values not significantly different at P < 0.05 for each species. U.C = upper cuticle; U.E = upper epidermis; P = palisade mesophyll; S = spongy mesophyll; L.E = lower epidermis; L.C = lower cuticle.

described earlier. For example in wet season the thickness of lower epidermis in *E. hirta* reduced from rural area with mean value of 4.25 ± 1.07μm to sub-urban with mean of 3.55 ± 1.15 μm and urban with mean of 1.90 ± 0.10 μm, but the reduction from rural to sub-urban location was not significantly different (P < 0.05). However, the mean value of the two sites are significantly different (P < 0.05) from that of the urban area. In addition, for lower cuticle, there was a reduction in the

thickness in *P. amarus* from 2.35 ± 0.49 µm in the rural area to 1.70 ± 0.47 µm in the sub-urban and 1.30 ± 0.47 µm in the urban site. While the reduction from rural to other areas was significant ($P < 0.05$) that of sub-urban to urban area was not ($P < 0.05$). Details of the result are presented in Table 5 for the dry season and Table 6 for the wet season.

Discussions

Previous study (Ekpemerechi *et al.,* 2014) established that the plants in each of the three sites investigated in this study grow under similar climatic conditions. The three sites however exhibit different microclimatic properties and most importantly pollution levels. The study suggested that the most influential factor which could have generated variations among the plants species in this case is the pollution levels. This position has been previously canvassed by many reports such as Agrawal (1985), Kalandadze (2003), Uaboi-Egbenni *et al.* (2009) and Stevovic *et al.* (2010) among others. These reports show that emission of various pollutants to the environment from automobiles exhaust, industrial production activities and other anthropogenic activities inflicts varying degrees of morphological and physiological damage to plants growing in such environments.

Earlier report on changes in leaf morphology of the selected plants species with respect to the level of pollution in various study sites (Ekpemerechi *et al.,* 2014) suggests changes may have taken place in the internal tissues of respective plants. The corresponding changes which occur in internal tissues in the current study could best be used to explain the mechanism that underlie reported changes in the morphology of these plants species with respect to variation in the level of pollution in their respective areas. For instance, the data from this study showed that in all the species investigated, the number of stomata or stomata frequency (Tables 1–2) increased significantly ($P < 0.05$) while the size of stomata (Tables 3-4) decrease significantly ($P < 0.05$) in polluted area (sub-urban and urban) when compared to unpolluted area (rural) in most cases.

A possible explanation of this response is that perhaps, the plants had to allocate more resources into the production of more stomata in order to access more CO_2 from the already polluted environment so as to be able to fulfill the required quantity of this gas needed for the all important photosynthetic process. Our findings is further substantiated by a number of earlier studies which report that stomatal area of plants exposed to auto-exhaust pollution exhibit different percentages of inhibition whereas the accompanying number of stomata, epidermal cells and stomata indices showed stimulation (Abdulmoniem, 2011). In addition, the data presented in this study is also supported by those of Tiwari *et al.* (2006) and Lakshmi (2010) who obtained a decrease in the sizes of stomatal opening and an increase in the frequency of the epidermal tissues and stomata in response to environmental pollution.

Another salient outcome of this study is with respect to the internal tissues of the leaves of all investigated species which equally showed a significant reduction (*P*

< 0.05) in the size of the tissues investigated. This reduction occurs in plants from polluted areas when compared to those from unpolluted area (Tables 5–6). It should be noted that these responses still vary among species, which underscores the established fact that responses of plants to environmental pollution is species-specific (Liu & Ding, 2008). For example, *F. virosa* showed a higher response in the cuticular reduction while species such as *A. cordifolia, E. hyssopifolia* do not show significant reduction ($P < 0.05$) in this tissue especially during dry season.

Reduction in the thickness of cuticles observed in this study is a response of plants to exposure of the leaves to pollutants since it is the first tissue exposed to pollutants among all other plants tissues. This is stressed by Baker *et al*. (1986) that plant cuticles, which are the main barriers between the interior and the outer environment of the leaf, remain in continuous contact with air pollutants and are responsive to changes in the environment. Plant cuticle is known to normally appear crystalline and soft (Shepherd & Griffiths, 2006) however these features can be altered by the impact of pollutants. Therefore, any specific or predictable alteration in cuticle due to air pollutants could serve as diagnostic marker as well as a response of the affected plant to levels of air pollution exposures.

Giri *et al*. (2013) reports that exposure to air pollution results in reduction of the total chlorophyll contents in the leaves of the affected plants. Earlier, Agrawal & Deepak (2003) have shown in their studies with soybean that a significant decrease in chlorophyll content and photosynthetic rate occur when exposed to high levels of air pollutant. Since chlorophyll is a pigments situated primarily in the mesophyll tissues of leaves (Pandey, 2012), any alteration to mesophyll tissues of a plant is expected to have corresponding effects on the chlorophyll content of such plants. From this study however, the mesophyll cells (palisade and spongy) of most of the plant species studied were observed to become flattened (data not shown) and significantly reduced ($P < 0.05$) in polluted sites in comparison to unpolluted site (Tables 5–6). This observation is apparently due to exposure to the pollutants that abound in the environment.

Interestingly, while *E. hyssopifolia* and *C. lobatus* showed a high level of significant reduction ($P < 0.05$) in mesophyll cells size among others, *A. cordifolia*, *E. hirta*, *M. esculenta* apparently do not show such significant reduction. These results further emphasise that the response of plants to changes in environmental conditions whether anthropogenic or otherwise is species-specific. This specificity expressed by the investigated plant species equally suggests that those that show significant response are species that could be considered to be susceptible or threatened while those with non-significant response could be said to be tolerant or stable with their exposure to these pollutants. This view is corroborated by reports of Iqbal (1985), Szabo *et al.* (2006) and Stevovic *et al.* (2010) who observed changes in shape and structure as well as reduction in the size of mesophyll cells as a result of pollution.

In conclusion, the data presented in this

study suggest that the responses of the leaf anatomical characters observed among the studied species are as a result of the differences in the pollutant levels in the three study sites. The responses in the anatomical characters are associated with the reported responses in the morphological attributes of the same plant species (Ekpemerechi *et al.,* 2014). This study has once again, successfully established the usefulness of anatomical studies in explaining the mechanism underlining the morphological responses especially to air pollution in plants. We are not oblivious of the fact that there may exist a wide range of other uncontrollable factors (weather condition, soil factor, parasites, to mention a few) which may play critical role in this. However, this present study provides an important contribution to knowledge which may compliment further studies on impact of environment on plants.

References

Abdulmoniem M. A. (2011). Effects of Auto-exhaust pollution on the Micro-Morphology and Leaf Epidermal Features of Ornamental Plants in Khartourn, Sudan. *Journal of Applied Sciences Research* **7**(3): 270–273.

Adedeji O. and **Jewoola O. A.** (2008). Importance of leaf epidermal characters in the Asteraceae family. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca.* **36**(2): 7–16.

Agrawal M. (1985). Plant factor as indicator of SO_2 and O_3 pollutants. Proceedings International symposium on Biological Monitoring of the state Environment (Bio-indicator), Indian National Science Academy. pp. 225–231. New Delhi.

Agrawal M. and **Deepak S. S.** (2003). Physiological and biochemical responses of two cultivars of wheat to elevated levels of CO_2 and SO_2, singly and in combination. *Environmental Pollution* **121**: 189–197.

Baker E. A. and **Hunt G. M.** (1986). Erosion of waxes from leaf surfaces by stimulated acid rain. *New Phytologist* **102**:161–173.

Dutta A. C. (2005). Botany for Degree Students. (6th Ed.). Oxford University Press. India.

Ekpemerechi S. E., Lala M. A., Jimoda L. A., Odiwe A. I. and **Saheed S. A.** (2014). Effect of air pollution on the foliar morphology of some species in the family Euphorbiaceae in Southwestern Nigeria. *Journal of Science and Technology* **34**(1): 21–29.

Emberson L. D., Ashmore M. R., Murray F., Kuylenstierna J. C. I., Percy K. E., Izuta T., Zhen G. Y., Shimizu H., Sheu B. H., Liu C.P., Agrawal M., Wahid A., Abdel-latii N.M., Van-Tienhoven M., de Bauer L.I. and **Domingos M.** (2001). Impacts of air pollutants on vegetation in developing countries. *Water, Air and Soil Pollution* **130**(1-4): 107–118.

Fenger J. (1999). Urban air quality. *Atmospheric Environment* **33**: 4877–4900.

Fuhrer J., Skarby L. and **Ashmore M. R**. (1997). Critical levels for ozone effects on vegetation in Europe. *Environmental Pollution* **97**: 91–106.

Giri S., Shrivastava D., Deshmukh K. and **Dubey P.** (2013). Effect of Air Pollution on Chlorophyll Content of Leaves. *Current Agriculture Research Journal* **1**(2): 93–98.

Gupta M. C. and **Ghouse A. K. M,** (1988). Effects of coal smoke pollutants from different sources in the growth, chlorophyll content, stem anatomy and cuticular trait of *Euphorbia hirta. L* Environ Pollut, **47**:221–230.

Heck W. W., Adams R. M., Cure W. W., Heagle A. S., Heggestad H. E., Kohut R. J., Kress L.W., Rawlings J. O. and **Taylor O. C**. (1983). A re-assessment of crop loss from ozone. *Environ. Sci. Tech*. **17**:573–581.

Illoh H .C. (1995). Foliar epidermis and petiole anatomy of four species of *Celosia L*. in Nigeria. *Feddes Repertorium* **106** (1–2): 15–23.

Iqbal M. Z. (1985). Cuticular and anatomical studies of white clover leaves from clean and air-polluted areas. *Poll Res.* **4**: 59–61.

Ivanescu L. and **Gosti I.** (2007). Structural and micro-morphological changes in leaves of *Salix alba* under air pollution effect. International *Journal of Energy and Environment.* **4**(1): 219–221.

Jahan S. and **Iqbal M .Z.** (1992). Morphological and Anatomical Studies on Leaves of Different Plants Affected by Motor Vehicle Exhaust. *Journal of Islamic Academy of Sciences* **5**: 21–23.

Kalandadze B. (2003). Influence of the ore mining and processing enterprise on soil types in adjoining areas. *Agron. Res.* **1**: 131–137.

Kovacic S. and **Nikolic T.** (2005). Relations between *Betula pendula* Roth. *(Betulaceae)* leaf morphology and environmental factors in five regions of Croatia. *Acta Biol. Cracov* **47**: 7–13.

Lakshmi B. O. (2010). Environmental pollution-Biological Risks-Oxidative Stress and Natural Antioxidant Principles. Paper presented in the sixteen NCA National symposium on applications of biotechnology in the environment management and medicine. p. 19-21. Davangere, India.

Liu Y.J. and **Ding H.** (2008).Variation in Air pollution Tolerance Index of Plants Near a steel Factory: Implication for Landscape-plant Species Selection for Industrial Areas. *WSEAS Transactions on Environmental Development* **4**: 24–32.

Ninoval D. J., Dushkova P. I. and **Kovacheva G. V.** (1983). Anatomical, Morphological studies of *Platanus acerifolia* at various degrees of air pollution. *Ekologiya* (Sofia) **6**: 35–47.

Ogundare C. S. and **Saheed S. A.** (2012). Foliar epidermal characters and petiole anatomy of four species of *Citrus* L. (RUTACEAE) from south-western Nigeria. *Bangladesh Journal Plant Taxonomy* **19** (1): 25–31.

Olorode O. (2012). Taxonomy of West African Flowering Plant. The Book Project, Obafemi Awolowo University, pp65-72. Ile-Ife, Nigeria.

Pandey B.P. (2012). Plant Anatomy. Chand S. and Company LTD, Ram Nagar, pp 295–315. New Delhi, India.

Pandey S., Kumar N. and **Kushwahar R.** (2006). Morpho-anatomical and physiological leaf traits of two alpine herbs, *Podophyllum hexandrum* and *Rheum emodi* in the western Himalaya under different irradiances. *Photosynthetic* **44**: 11–16.

Qadir S. A. and **Iqbal M. Z.** (1991). Growth of some plants raised from polluted and unpolluted seeds. *International Journal of Environmental Studies* **39**: 95–99 .

Saheed S. A. and **Illoh H. C.** (2010). A taxonomic study of some species in *Cassinae* (Leguminosae) using leaf epidermal characters. *Not. Bot. Hort. Agrobot. Cluj* **38**(1): 21–27.

Salgare S. A. and **Rawal M.** (1990). Effect of auto-exhaust pollution at Andheri (West), Bombay on anatomy of some cultivated plants (monsoon season). *Journal of Ecobiology* **2**(4):273–280.

Shepherd T. and **Griffiths D. W.** (2006). The effects of stress on plant cuticular waxes. *New Phytologist* **171**: 469–499.

Singh N., Yunus M., Srivastava K., Singh S. N., Pandey V., Misra J. and **Ahmad K. J**. (1995). Monitoring of auto-exhaust pollution by road side plants. *Env. Mont. Ass.* **34** (1):13–25.

Sodnik H., Skrezyna J. J. and **Staszkiewicz J.** (1987). The effect of industrial pollution in Walbrzych (Poland) on the size and shape of leaves of selected species of trees. Rocz. Sekc. Dendrol. *Pol. Tow. Bot.* **36**: 17–34.

Stevovic S., Vesna, S. M. and **Dusica C.** (2010). Environmental impact on morphological and anatomical structure of Tansy. *African Journal of Biotechnology* **9**(16): 2413–2421.

Szabo Z. K., Papp M. and **Daroczi L.** (2006). Anatomy and morphology of five *Poa* species. *Acta Biol. Cracov*. **48**: 83–88.

Tiwari S., Agrawal M. and **Marshall F. M.** (2006). Evaluation of ambient air pollution impact on carrot plant at a sub-urban site using open top chambers. *Environmental monitoring and Assessment* **119**: 15–30.

Uaboi-Egbenni P. O. Adejuyitan O. E., Sobande A. O. and **Akinyemi O.** (2009). Effect of industrial effluent on the growth and anatomical structures of *Abelmoschus esculentus* (okra). *African Journal of Biotechnology* **8**: 3251–3260.

Watson L. and **Dallwitz M. J.** (1992). onwards. The families of flowering plants: descriptions, illustrations, identification, and information retrieval. Version: 11th May 2015. http://delta-intkey.com' (Accessed 24th May, 2016).